Evidence of Debromination of Decabromodiphenyl Ether (BDE-209) in Biota from a Wastewater Receiving Stream

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Decabromodiphenyl ether (BDE-209) is a high production volume flame retardant. To date, regulation and control of its environmental release have been minimal. Once in the environment, BDE-209 may encounter conditions favoring debromination, potentially forming congeners with greater toxicity, bioaccumulation potential, and persistence. However, (photolytic and in vivo) debromination has only been demonstrated under laboratory scenarios. To examine whether debromination was likely in the field, PBDE congener profiles were tracked from a wastewater treatment plant (sludge) to receiving stream sediments and associated aquatic biota. BDE-209 and 23 additional PBDEs were detected. Sludge congener profiles resembled the commercial penta- and deca- formulations, suggesting minimal -209 debromination during wastewater treatment. Similar profiles were observed in surficial sediments at the outfall and downstream. However, sunfish (*Lepomis gibbosus*), creek chub (*Semotilus atromaculatus*), and crayfish (*Cambarus puncticambarus sp. c*) collected near the outfall contained tri- through deca-PBDEs, including congeners not detected in the commercial deca- mixture, sludges or sediments (BDE-179, -184, -188, -201, and -202). A previous in vivo laboratory study identified these as -209 debromination products. This supports the hypothesis that metabolic debromination of -209 does occur in the aquatic environment under realistic conditions. Hence assessments that assume no BDE-209 debromination may underestimate associated bioaccumulation and toxicity attributable to the less brominated congeners produced.

Introduction

Polybrominated diphenyl ethers (PBDEs) are widely used flame-retardants. They have become globally distributed, akin to other persistent organic pollutants such as PCBs. Although, BDE-47 and -99 are the most commonly reported congeners, researchers have observed nearly 40 additional congeners in various biological matrixes. Of these congeners, 13 have not been reported as components of common commercial PBDE formulations (*1*). Therefore they may result from debromination of higher brominated PBDEs. In contrast, deca-, the PBDE product of greatest historical demand and the only formulation still in production in North America and Europe, has been reported with less frequency (*2*, *3*). Its primary constituent, BDE-209, has been detected at high concentrations in sediments near points of initial release (*4*). However, with the exception of birds of prey (*5*), reported concentrations in biota have been comparatively low. This has been attributed to its reportedly low bioavailability and tendency to strongly bind to sediment and soil.

BDE-209 can debrominate when dissolved in certain solvents and subjected to UV irradiation (*6*). Major homologues that have been observed include tri- through octa-PBDEs and a host of brominated dioxins and furans. Photolytic debromination of BDE-209 associated with artificial and natural sediment, soil, and sand has also been reported (*7*−*9*). Studies have observed an increase in the lower brominated (hexa- through nona-) PBDEs. Söderström et al. (*8*) identified BDE-47, -99, and -154 on silica gel originally amended with BDE-209. They also reported -209 dissolved in toluene and exposed to UV light generated BDE-99, -100, -153, and -154. Recently, Ahn et al. (*9*) exposed -209 adsorbed matrixes made up of clay minerals, metal oxides, and sediment to UV irradiation, lamp and natural. Tri- to nona-PBDEs were detected after 3 days of lamp-irradiation and 14 days of sunlight-irradiation in two of the six matrixes (montmorillonite (2:1 clay mineral; smectite) and kaolinite (1:1 clay mineral)). However, BDE-47 and -99 were not detected. Longer half-lifes for BDE-209 adsorbed to more complex matrixes, i.e., natural sediment or soil than artificial ones, were also observed by Söderström et al. (*8*), and Ahn et al. (*9*). This was attributed to natural soil and sediment particles being more porous and organic carbon rich than artificial ones, providing UV-radiation shielding (*8*, *9*). Hua et al. (*10*) reported increasing humic acid contents deceased degradation rates for UV-irradiated BDE-209.

As much as 97% of the deca- product is formulated with plastics for electronic equipment, e.g., housings and wire coatings. This incorporation and the chemical properties of BDE-209, namely low water solubility (<20−30 μg/L) and vapor pressure (<10^{-6} mmHg at 20 °C) (*11*) retard its release and mobility. This also will limit its photodegradation and bioavailability potential. However, recent studies have shown that household dust can contain PBDEs, including BDE-209, at mg/kg levels (*12*, *13*). Also, industrial discharges may release deca-. According to the U.S. EPA Toxic Release Inventory (http://www.epa.gov/tri/ (*14*)), total U.S. industrial releases on and off-site to land (e.g., air, surface water, and landfills) of BDE-209 from 1988 to 2004 averaged over 500 metric tons (MT) per year. Approximately 90 MT more per year were released to wastewater treatment plants (WWTPs). Household waste (containing PBDE-laden dust) and other unquantified industrial activities may transfer additional PBDEs to WWTPs. PBDEs may then reach the environment sorbed to particulates in the aqueous effluent (*15*) or via land applied sewage sludge (*16*).

Few studies have examined the dietary uptake and biotransformation of BDE-209. Juvenile rainbow trout (*Oncorhynchus mykiss*) were fed cod chips spiked with BDE-209. BDE-209 was not detected in the fish, but BDE-47, -99, -153, and several nonspecified hexa- to nona-PBDEs were reported (*17*). Their concentrations in liver and muscle increased with length of exposure. BDE-153, -154, and an unidentified octa-PBDE were not detected in the original deca- mixture, indicating likely transformation of BDE-209. More recently, juvenile carp (*Cyprinus carpio*) were fed BDE-209 (>98% purity) spiked food for 60 days (*18*). At the end of this study

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BDE-209 was not detected in the carp tissues, however seven other congeners were observed and accumulated over time. Two apparent metabolites were identified as BDE-154 and -155, the remainder were only identified as to their degree of bromination, i.e., as penta- through octa-PBDEs. In another experiment, dietary exposure of carp to BDE-183 or -99-spiked food, resulted in apparent metabolic debromination of both congeners (*19*), i.e. conversion of BDE-99 to -47, and -183 to -154 and an unidentified hexa-PBDE. Approximately 10% of the original doses of BDE-99 and -183 were detected as BDE-47 and -154, respectively. The extent of metabolic debromination is likely species-dependent. This would account for the low contribution of BDE-99 (<0.1%) to the total PBDEs reported in carp from the Hyco River in Virginia (U.S.) (*20*). Similar PBDE contributions were also observed in feral carp and sediments collected from a heavily industrialized portion of the Llobegat River, Spain (*21*). In a follow-up to the carp exposure study, juvenile rainbow trout were exposed in the lab to -209 via the diet for 5 months (*22*). BDE-209 concentrated in the liver. Several hepta-, octa-, and nona-PBDEs congeners also accumulated in the trout's liver. To determine whether the observed debromination was a result of metabolism by the fish, liver microsomes were prepared from both carp and rainbow trout and incubated with BDE-209. As much as 22% of the BDE-209 mass was biotransformed, primarily to octa- and nona-PBDEs, in the trout liver. About 65% of -209 was transformed to hexa-PBDEs in the carp, indicating species-dependent metabolic debromination.

From these studies, it is apparent that in the laboratory BDE-209 can undergo photolytic and biological debromination. Hence, once BDE-209 is released to the environment, it may encounter conditions conducive to debromination, contributing to the environmental burdens of the now largely discontinued, lower brominated PBDEs. However, this phenomenon has not been previously documented in an actual environmental setting. While it appears that fish took up some BDE-209 from heavily spiked food in the laboratory, questions remain as to the extent to which BDE-209 in the environment is available for uptake. Here, we evaluated congener distributions of PBDEs in WWTP sludges, and downstream receiving water sediments and aquatic biota for evidence of potential debromination.

Experimental Section

Study Site. The U.S. EPA Toxic Release Inventory (TRI) lists U.S. industry reported releases (e.g., air emissions and discharges to surface water) and transfers (e.g., landfills and WWTPs) of high production volume chemicals. Of the PBDEs, deca- is the only PBDE product tracked in this way. According to the TRI, 27 U.S. facilities released more than 4.5 MT of BDE-209 in 2001 (*14*). The total 2001 releases from these facilities were approximately 500 MT, primarily from deca-chemical manufacturing and chemical waste management facilities. The fifth largest reported amount from an individual operation, 45 MT, was from a North Carolina (NC) plactic goods manufacturer. Reported discharges were reported to be via a wastewater treatment plant (WWTP), rather than directly to surface waters. Preliminary findings (October, 2002) revealed BDE-209 at 12 μg/L in the whole WWTP effluent, with trace levels (<20 ng/L) of BDE-47 and -99 (*23*).

The WWTP is an activated sludge-type secondary facility that releases an average of 2.1-MGD of treated effluent into Marlowe Creek, downstream from the City of Roxboro, NC. During dry months, this discharge is more than 99% of the total creek flow. Marlowe Creek flows into Storys Creek, which later enters Hyco River approximately 11 km downstream from WWTP outfall. The Hyco River flows northeast from North Carolina and enters the Dan River downstream from South Boston, Virginia. The headwaters of the Hyco are dammed to form the After Bay Reservoir located approximately 35 km upstream from the Dan River. Water released from the reservoir flows down the Hyco River through rural and agricultural areas.

Sample Locations. Samples (wastewater sludge, sediments and biota) were collected in Fall 2002 (Table ST1, Supporting Information) to determine if PBDEs used in local manufacturing were transferred for further treatment to the WWTP and released via its effluent. The extent of PBDE contamination from the WWTP outfall to a site 11 km downstream was also investigated. In Fall 2005 additional samples were collected at the outfall and several locations downstream (Table ST1) to ascertain current PBDE burdens and for evaluation of detailed congener profiles as evidence of environmental debromination.

Within WWTPs, PBDEs reside primarily sorbed to solids. Hence analysis of these burdens provides an estimate of the amount introduced to the facility. Accordingly, activated sludge (4 L) was collected in 2002 and 2005. To determine release of PBDEs to the effluent-dominated receiving stream, bed sediments and aquatic biota were also collected near the outfall. The sample site chosen, approximately 15 meters downstream from the outfall, was presumed well mixed. Surficial sediments (1 L) were collected here and minnow traps were placed on both sides of the stream nearby. Traps were emptied approximately 24 h after deployment, captured biota separated by species, and transferred to holding tanks for depuration (72 hours).

Surficial sediments (1 L) were also taken upstream and several locations downstream from the outfall. Once introduced into aquatic environments, wastewater particulates with associated hydrophobic PBDEs can settle out and contribute to sediments (*24*). Sites were distributed along approximately 11 kilometers of river system and were resampled in 2005.

Methodology. Whole biota was composited and homogenized and then freeze-dried. Sediments were freeze-dried and then sieved (2000 μm) to remove large debris. Sewage sludge was centrifuged, excess water decanted, and then freeze-dried. All samples were stored in glass jars with Teflon lids at <0 °C until they were analyzed.

For PBDE determinations, each sample (0.5 g sludge, 9−10 g biota, 20 g sediment, d.w.) was extracted by enhanced solvent extraction (Dionex ASE 200, Sunnyvale, CA). A surrogate standard (1 μg) 2,2′, 3,4,4′, 5,6,6′-octachlorobiphenyl (PCB-204) (Ultra Scientific, North Kingstown, RI) was added prior to the extraction. Each extract was purified by size exclusion chromatography, (SEC, Envirosep-ABC, 350 × 21.1 mm. column; Phenomenex, Torrance, CA). The post-SEC fraction of interest was reduced in volume, added to a 2 g silica glass column (Isolute, International Sorbent Tech., Hengoed Mid Glamorgan, UK) and eluted with 3.5 mL hexane, followed by 6.5 mL of 60:40 hexane/DCM. The second fraction, containing the PBDEs, was reduced in volume and solvent exchanged to hexane. Pentachlorobenzene (PtClb) and decachlorodiphenyl ether (DCDE) (Ultra Scientific, North Kingstown, RI) were added for retention time markers. DCDE was also used as an internal quantitation standard. (For further information on sample extraction see the Supporting Information.) Analytical blanks ($NaSO_4$) were also extracted and analyzed with each sample batch and all results corrected based on surrogate (PCB-204) recoveries (Tables 1 and 2). Method validation including matrix ($NaSO_4$, sediment, and biota) spiking information and results, as well as replicate analyses is included in the Supporting Information (Table ST2).

Compounds of interest in the purified extracts were separated by gas chromatography (GC), (6890N, Agilent Tech., Palo Alto, CA) equipped with an on-column injector, using a 30-m DB-5HT (0.25 mm i.d., 0.1 μm, J&W Scientific, Agilent

TABLE 1. PBDEs in Wastewater Sludge and Surficial Sediments Collected in 2002 and 2005.

	collected, November 2002						collected, November 2005					
		sediments (μg/kg, %TOC), km from outfall						sediments (μg/kg, %TOC), km from outfall				
congener	sludge (μg/kg, dw)	−0.2 km	0 km	1.3 km	5.6 km	10.8 km	sludge (μg/kg, dw)	−0.2 km	0 km	1.3 km	5.6 km	10.8 km
BDE-17	nd	nd	nd	nd	25	59	14	nd	nd	nd	nd	nd
−28	nd	nd	nd	74	137	98	78	nd	nd	nd	nd	nd
−49	98	105	nd	383	330	364	95	144	nd	nd	nd	nd
−71	51	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
−47	1670	266	1180	2540	3510	2910	822	964	754	2030	3580	231
−66	125	nd	nd	nd	161	107	55	51	nd	nd	nd	nd
−100	422	115	371	645	1410	1080	305	466	372	627	1180	107
−99	1660	482	1830	3200	5540	3990	918	2210	2010	3740	5410	337
−85	255	25	87	148	249	149	108	74	nd	150	238	nd
−154	295	53	nd	nd	465	303	136	nd	nd	nd	371	35
−153	488	192	375	432	941	556	187	251	nd	525	839	123
−138	nd	nd	nd	nd	119	68	nd	nd	nd	nd	nd	nd
−183	310	nd	nd	nd	249	125	89	nd	nd	nd	nd	nd
−197	993	nd	nd	nd	156	73	171	nd	nd	nd	388	nd
−203	1190	nd	434	386	322	166	220	nd	nd	nd	553	nd
−196	1600	nd	380	771	620	210	202	nd	nd	nd	1120	nd
sum, tri- through octa-BDEs (%)	**9160 (9.4%)**	**1240 (3.3%)**	**4660 (0.3%)**	**8580 (0.3%)**	**14200 (2.1%)**	**10300 (3.2%)**	**3400 (7.9%)**	**4160 (11.1%)**	**3140 (1.6%)**	**7070 (0.3%)**	**13700 (0.6%)**	**833 (0.3%)**
−208	726	nd	3530	nd	577	nd	295	nd	nd	nd	1690	375
−207	1340	nd	5810	6660	2630	945	276	nd	nd	nd	6520	544
−206	27400	nd	67700	84000	24300	10900	1490	nd	11200	31700	35500	3120
−209	58800	36800	1630000	3150000	642000	300000	37400	33300	181000	2310000	2390000	247000
sum of nona- through deca-BDEs (%)	**88300 (90.6%)**	**36800 (96.7%)**	**1710000 (99.7%)**	**3240000 (99.7%)**	**670000 (97.9%)**	**312000 (96.8%)**	**39500 (92.1%)**	**33300 (88.9%)**	**192200 (98.4%)**	**2340000 (99.7%)**	**2440000 (99.4%)**	**251000 (99.7%)**
total PBDEs	**97400**	**38000**	**1710000**	**3250000**	**684000**	**322000**	**42900**	**37500**	**195000**	**2350000**	**2450000**	**252000**
%REC, PCB-204	73%	85%	106%	88%	81%	82%	62%	76%	69%	69%	73%	68%
%TOC	28.2	0.12	0.07	0.07	0.89	0.76	25.3	0.12	0.06	0.08	0.09	0.59

TABLE 2. PBDEs (μg/kg, % Lipid) in Biota Composites, Collected in 2002 and 2005

	collected, November 2002			collected, November 2005
congener	chub ($n = 6$)	crayfish ($n = 5$)	sunfish ($n = 13$)	sunfish ($n = 22$)
BDE-17	nd	nd	32	29
−28	285	nd	246	179
−49	618	nd	1300	855
−71	nd	nd	nd	541
−47	17200	4110	11600	7600
−66	nd	nd	1670	952
−100	3460	nd	2340	1820
−99	nd	3560	13300	5220
−85	725	nd	496	229
−154	2610	nd	2290	1880
−153	918	767	3110	2420
−188	1450	nd	884	289
−184	nd	nd	360	164
−179	1140	nd	166	137
−183	nd	nd	83	77
−202	895	87	747	243
−201	129	78	773	335
−197	nd	43	193	86
−203	117	132	74	20
−196	45	200	65	28
−208	103	143	201	67
−207	79	1920	276	73
−206	94	2650	411	133
−209	nd	21600	2880	nd
total PBDEs	**29900**	**35300**	**43500**	**23377**
%rec. PCB-204	60%	81%	90%	72%
%lipids	21.9	5.2	10.5	17.2

Tech.) column. Ion fragmentation spectra used for compound identification were produced by electron-capture negative ionization (ECNI) and electron ionization (EI) (JMS-GC Mate II, JEOL, Peabody, MA). Recently, it has been reported that 64 PBDEs (mono- through deca-PBDEs) can be reliably analyzed using a single 30-m DB-5HT (*1*). However, poor chromatography (unresolved peaks) was observed for the biota and sludge samples, perhaps due to column overloading by coextractives. Accordingly, these samples (biota, sludge, and calibration standards) were reanalyzed using a pressure pulse split/splitless injector, which greatly improved the chromatography. This injection technique has been previously reported suitable (minimum -209 thermal degradation) for mono- through deca-PBDE analysis (*25*).

The target analytes were first detected and quantified by ECNI-SIM using m/z 79 ($[^{79}Br]^-$), 81 ($[^{81}Br]^-$) for the PBDEs and m/z 35 ($[^{35}Cl]^-$), 37 ($[^{37}Cl]^-$) for the internal, surrogate, and retention time standards. Five-point quantification curves (0.998 minimum r^2 acceptance value) were generated by analyzing dilutions of a PBDE standard (Wellington Laboratories Inc., Ontario, Canada) containing 27 PBDEs ranging from mono- to deca-PBDEs. An additional seven PBDEs previously identified as potential debromination products (*22*) (Table ST3) were also analyzed. Mono-through nona-BDE concentrations ranged from 10 to 1000 pg and BDE-209 ranged from 50 to 5000 pg on-column for both injection programs. To assist in sample compound identification, relative retention indices (RRI) were calculated for both on-column and split/splitless injector programs for each of the analytical standards, as previously reported (*1*).

Along with RRI, fragmentation patterns and isotope intensity spectra produced by ECNI (scan range 10−550 *m/z*) and EI (scan range 50−1000 *m/z*, scan time 0.30 s., electron energy 70 eV) were used to identify PBDEs. (The previously stated GC conditions were used for both ECNI and EI analyses.) The predominant ions generated in ECNI spectra of PBDEs are 79 and 81 *m/z*. However, cleaving at the ether bond has also been observed for hepta-, octa-, nona-, and deca-PBDEs (*1*). These produced spectra with ion clusters centered around 328 and 330 m/z for $[C_6Br_3H_2O]^-$, 408 m/z for $[C_6Br_4HO]^-$ and 486 and 488 m/z for $[C_6Br_5O]^-$. For PBDE identification, bromine distributions between the two benzene rings can be determined for hepta- through deca-PBDEs by examining these fragments (*1*). In EI mode, the dominant ion clusters are centered on the molecular ion $[M]^+$ and the loss of two bromines $[M-Br_2]^+$ (*1*). Table ST3 of the Supporting Information contains the predominant ions products produced by ECNI and EI modes for compound identification used in this study.

Results and Discussion

Wastewater Sludge. Tri- through deca-PBDEs were detected in the 2002 and 2005 WWTP sludges, indicating the potential for environmental exposure through PBDE-laden effluent particulates. It has been estimated that >90% of the PBDEs entering a WWTP will ultimately reside within the sludge, with the remainder released via its effluent, primarily sorbed to suspended particulates (*15*, *26*). A total of 17 PBDEs were identified in the 2002 and 18 in the 2005 sample (Table 1). The major PBDE congener in both was BDE-209 (58 800 and 37 400 μg/kg (d.w.) for the 2002 and 2005 samples, respectively), followed by BDE-99 and -47. For the tri- through octa-PBDEs, major constituents of the penta- and octa-formulations, a 55% lower sludge concentration was observed in 2005 than 2002 (Table 1). This reduction may relate to the December 2004 cessation in the U.S. manufacture of these formulations (the 2005 sample was collected in November).

The EPA TRI indicated substantial delivery of deca- to this WWTP from a major manufacturer of plastic goods. Penta- and octa- were also likely utilized to some extent but, not being high production volume chemicals, were not required to be reported in the TRI. It has also been hypothesized that releases from finished products in use, in addition to manufacturing, may contribute PBDEs to wastewaters. For example, indoor dust can contain mg/kg PBDE burdens (*27*), which can eventually enter household waste streams. Also, as products containing PBDEs age, their release may increase (*27*).

According to the TRI, 2287 kg in 2002 and 1692 kg in 2004 of BDE-209 were transferred to the WWTP examined. Prior to 2002, the TRI suggested it received over 34 000 kg of BDE-209 per year. Maximum reported transfer, 113 826 kg, occurred in 1994 (Figure SF1, Supporting Information). However, since 1999 transfers decreased 10-fold and by 26% between 2002 and 2004. This reduction may be reflected in the 41% lower -209 sludge burden in 2005 than in 2002 (Table 1). Also apparent in these sludges was a decline of the three nona- (BDE-206, -207, and -208) and three octa-BDEs (BDE-196, -203, and -197) (Table 1). It has been previously reported that -209 can debrominate under anaerobic conditions. Gerecke et al. (*28*) incubated -209 with sewage sludge collected from an anaerobic digester. They reported the appearance of several degradation products (two nona-PBDEs (BDE-207, −208) and three octa-PBDEs (tentatively identified as BDE-196, -198/203, and -197). However, no other debromination products were observed. Debromination of -209 to PBDEs with <7 bromines was also not observed in sludge collected from different stages of wastewater treatment from 11 German WWTPs (*29*).

PBDEs have been previously detected in other WWTP sludges. Hale et al. (*30*) reported a maximum BDE-209 concentration of 4890 μg/kg, mean 1010 μg/kg (d.w.), in 11 sludges (biosolids) collected from four different regions of the U.S. They also reported five additional PBDEs (BDE-47, -100, -99, -153, and -154) ranging from 1100 to 2290 μg/kg (d.w.). These congeners contributed an average 75% of the

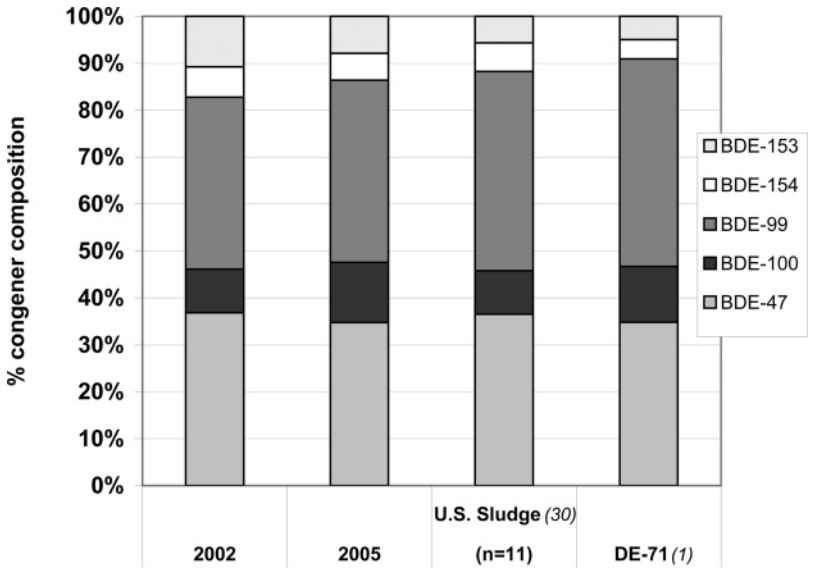

FIGURE 1. Distributions of tetra- through hexa- brominated congeners in Roxboro WWTP sludge (2002 and 2005) compared to U.S. sludges and penta-formulation (DE-71).

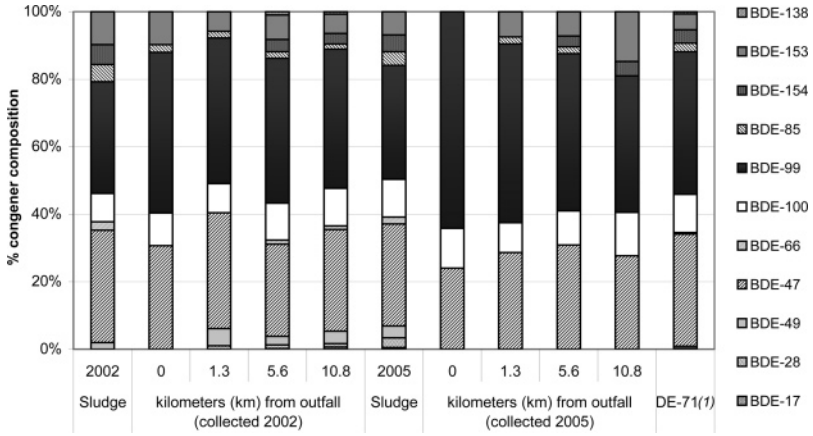

FIGURE 2. Sediment and sludge congener profiles (tri- through hexa-PBDEs), 2002 and 2005.

total PBDE sludge concentration, with the remainder predominantly from BDE-209. In one sludge, -209 contributed 70% of the total PBDEs detected. For the Roxboro WWTP sludges, BDE-209 was the major PBDE congener detected, constituting 60 and 87% of the total, respectively (Table 1). The BDE-209 concentrations here exceeded the previous survey's maximum reported -209 values by 10-fold, likely due to its usage by local plastics manufacturers. However, the sum of BDE-47, -100, -99, -153, and -154 for the 2005 Roxboro sludge (2370 μg/kg, d.w.) was similar to this survey's maximum value. The 2002 sludge burden (4540 μg/kg, d.w.) was only twice this concentration. The congener profiles in the Roxboro sludges approximated that of intact penta-technical formulations, as previously reported for other U.S. samples (*30*) (Figure 1). This suggests that BDE-209 debromination is unlikely to be a major contributor to the lower brominated PBDEs seen in Roxboro sludges.

Sediments. Surficial sediments were also collected in 2002 and 2005. Major PBDE congeners detected were BDE-209, -206, -99, and -47. A total of 20 tri- to deca-BDE congeners were observed in the 2002 samples (14 in 2005) (Table 1). BDE-209 contributed >89% of total PBDEs in each of the sediments, followed by the nona-PBDEs which constituted 3−10%. Most sediment also contained tri- through octa-PBDEs. However, their total contributions were an order of magnitude lower than that of -209 (Table 1). Interestingly, BDE-206 was the second most abundant PBDE detected, up to 84.0 mg/kg, normalized to TOC content, and its concentrations exceeded those of both BDE-47 and -99 in 8 out of 10 samples. Total PBDEs levels were actually greatest several kilometers downstream from the outfall and were still detectable at the furthest downstream collection point (10.8 km) from the outfall, where Storys Creek meets the Hyco River. Concentrations were lower upstream of the outfall (−0.2 km) in 2002 and 2005, 38.0 and 37.5 mg/kg (TOC basis), respectively (Table 1). These values indicate additional PBDE releases via urban runoff or other sources. Maximum sediment concentrations in 2002 and 2005 were detected between 1 and 6 km downstream of the outfall (3250 mg/kg and 2450 mg/kg, respectively). Further down stream (10.8 km) levels dropped (322 and 252 mg/kg, 2002 and 2005, respectively), but still exceeded by 10-fold by those upstream of the outfall (Table 1). The 2005 (5.6 km) sediment exhibited a 3-fold greater concentration than the 2002 sampling (Table 1). This may indicate variations in WWTP discharge, relocation of historic PBDE reservoirs in the river system, or simply heterogeneity within the sampling area.

BDE-209 has been shown to undergo photolytic (6, 7, 8, and 9) and microbial debromination (*31*) under selected laboratory conditions. Debromination could generate degradates with increased toxicological potential. Sediments contained similar concentrations of tri- through octa-BDEs in the 2002 and 2005 sediment sets. An exception was the 2005 sample taken 10.8 km downstream of the outfall, where

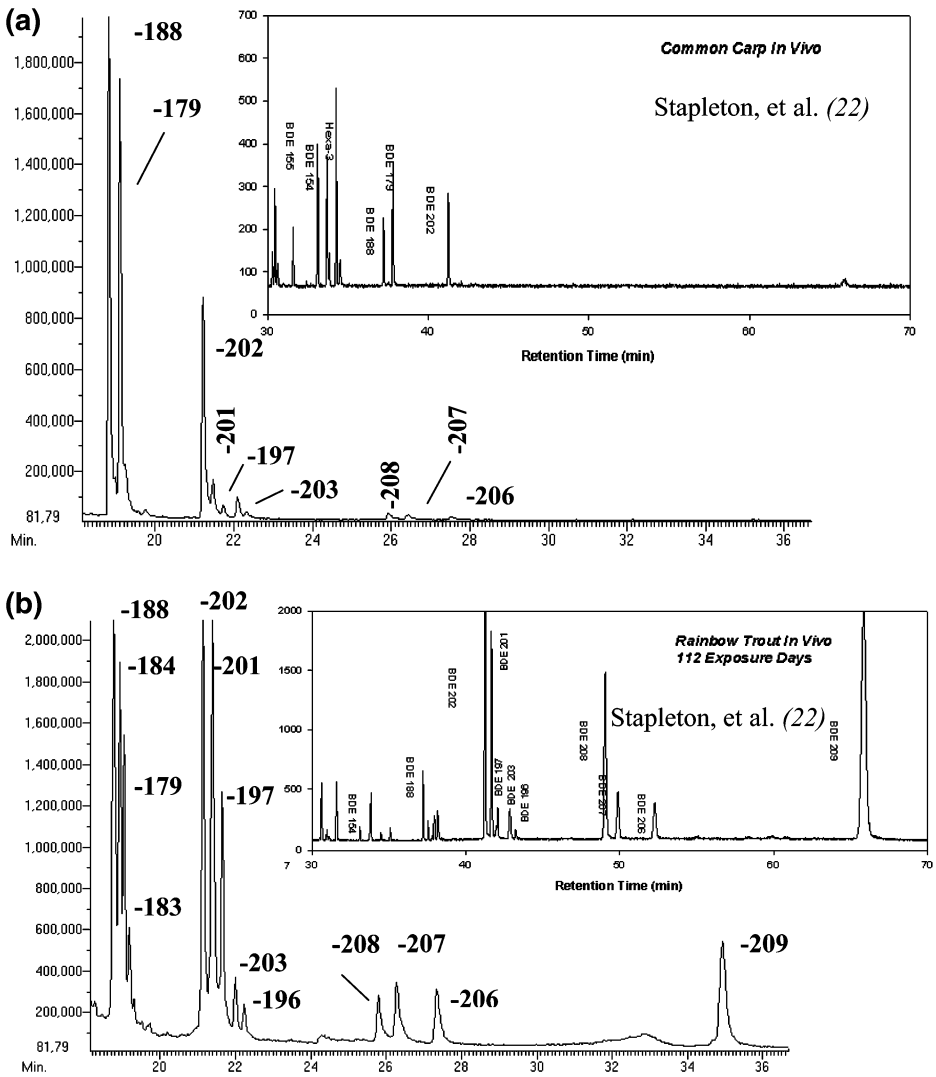

FIGURE 3. (a) ECNI-SIM (*m/z* 79, 81) chromatogram of PBDEs in Roxboro creek chub compared to common carp BDE-209 laboratory exposure study. (b) ECNI-SIM (*m/z* 79, 81) chromatogram of PBDEs in Roxboro sunfish compared to rainbow trout BDE-209 laboratory exposure study.

a 92% decrease was detected (Table 1). Concentrations of tri- through octa-BDEs also increased with distance from the outfall (Table 1). Maximal concentrations for these congeners in 2002 and 2005 in sediments 5.6 km downstream were 14.2 and 13.7 mg/kg (%TOC), respectively. Nona- and deca-BDEs levels in 2002 showed high levels at the outfall and a maximum at 1.3 km downstream (Table 1). In 2005, the 0 and 1.3 km downstream levels were lower, but the 5.6 km sample was higher. One explanation would be a decrease in releases from the WWTP in later years or downstream transport of an episodic deca release. Periodic releases of untreated sewage from this WWTP have been reported due to storm events. Further downstream (10.8 km) tri- through octa-BDEs concentrations were lower, while the BDE-209 burden was comparable to the 2002 levels. Regardless, tri- through octa-PBDEs only accounted for 3.2% or less of total PBDEs downstream from the outfall (Table 1). Others have also reported similar PBDE contributions in -209 rich sediments (>7000 μg/kg, d.w.) and attributed the less brominated congeners to commercial penta- formulations (*32*, *33*). Congener profiles for the tri- through hexa-PBDEs were comparable for Marlowe/Storys sediments collected in 2002 and 2005, at the outfall and 1.3, 5.6, and 10.8 km downstream (Figure 2). BDE-47 and -99 were the major PBDEs detected and profiles generally resembled the penta-technical formulation (e.g., DE-71). Congener profiles were also similar to sludge collected from the Roxboro WWTP (the probable source of PBDEs to Marlowe/Storys Creek), indicating that minimal -209 debromination has occurred in surficial sediments along the 10.8 km creek system.

Biota. Two fish species (sunfish (*Lepomis gibbosus*, composite of 13 individuals) and creek chub (*Semolilus atromaculatus,* composite of six individuals)) and a crustacean (crayfish (*Cambarus puncticambarus sp.c*, composite of five individuals)) were collected at the outfall in 2002. In 2005, only sunfish (composite of 22 individuals) were available there. A total of 23 PBDEs were detected in the biota samples, ranging from tri- to deca- PBDEs (Table 2). BDE-47, -153, four octa-BDEs (BDE-202, -201, -203, and -196) and three nona-BDEs (BDE-208, -207, and -206) were detected in each of the biota samples. BDE-209 was observed in two of the fish samples and was only exceeded concentration-wise by the -47, -99, and -153 (Table 2). BDE-209 was also detected in the crayfish composite (21 600 μg/kg, l.w.). Interestingly, this is an order of magnitude higher than the sunfish sample (2880 μg/kg, l.w.) (Table 2). To our knowledge, this is the first report of PBDEs in crayfish. PCBs bear some structural similarities to PBDEs and have been previously reported from the river Meuse (Netherlands) in crayfish (*Orconectus limosus*). Congener profiles followed those in fish, favoring tri- through hexa-PCBs (*34*). Holmqvist et al. (*35*) compared PCBs in crayfish (*Pacifastacus leniuscukus*) from Swedish streams and lakes and found greater variability in total PCBs in stream crayfish. This was attributed to crayfish being omnivorous

and hence more influenced by diverse contaminant sources within their catchment. BDE-209 accounted for 95% of the PBDEs in sediments where the Roxboro crayfish were collected. Sediment-associated dietary items likely contributed to the high BDE-209 contribution, 59% of total PBDEs. Hence benthic invertebrates could serve as a route of -209 exposure to aquatic and terrestrial predators.

BDE-47 was the second most dominant congener reported. It was lowest in crayfish (4110 ug/kg l.w.) and highest in chub (17 200 μg/kg, l.w). BDE-47 has previously been reported as the most abundant congener in Virginia fish, ranging from 45% of total PBDEs in channel catfish to 74% in carp (*20*). Congener profiles for tetra- through hexa-BDEs in the two Roxboro sunfish composites were more comparable to the penta- technical mixture, i.e., exhibited similar BDE-47 and -99 contributions.

The congener profile for the chub sample was devoid of BDE-99 and -154 concentrations were elevated compared to -153. This same congener profile was previously reported in common carp (*Cyprinus carpio*) collected from the Dan and Roanoke Rivers (*20*). Stapleton et al. (*19*) exposed common carp to BDE-99 and -183 via the diet and reported significant conversion of BDE-99 to -47 and -183 to -154. Neither congener (BDE-99 or -183) were detected in the Roxboro chub, but were observed in surrounding sediments and in other fish species at this site. The creek chub (*Semotilus atromaculatus*) and common carp (*Cyprinus carpio*) both belong to the same family (*Cyprinidae*) and may metabolize PBDEs similarly. Hence varying biotransformation capacities may contribute to the different PBDE congener profiles observed between the various fish species, as well as the crayfish.

Although BDE-209 was only detected in the 2002 sunfish, both sunfish composites did contain three nona-PBDEs. They also contained five octa-PBDEs and four hepta-PBDEs. The chub composite contained three nona-, four octa-, and two hepta- PBDEs. Of these, two octa- (BDE-201, -202) and three hepta- (BDE-188, -184, and −179) congeners were not detected in either the sludge or sediment samples. Therefore, these conceivably could be metabolic debromination products of the higher brominated PBDEs (e.g., BDE-209). In a follow-up to their initial carp exposure study, Stapleton et al. (*22*) conducted a BDE-209 dietary exposure experiment using juvenile common carp and rainbow trout (*Oncorhynchus mykiss*). After 60 days of exposure (112 days for the trout), carp whole body homogenates were extracted and analyzed for PBDEs. BDE-209 was not detected in the carp sample. However, one octa- (BDE-202), two hepta- (BDE-179 and -188) and three hexa-PBDEs (BDE-154, -155 and one unidentified hexa-BDE) were reported, indicating that carp can metabolize -209 to lower brominated diphenyl ethers. As noted previously, common carp may metabolize PBDEs similarly to creek chub. Chromatograms of the lab-exposed carp and the chub from Marlowe/Storys Creek exhibit comparable hepta- through deca- congener patterns (Figure 3a). PBDEs present were identified as one octa- (BDE-202) and two hepta- (BDE-188 and -179). These were not detected in the sediments or sludge.

In the Stapleton et al. (*22*) rainbow trout dietary BDE-209-exposure study, -209 was detected, as well as several hepta- (BDE-188, -184, 179, and -183), octa- (BDE-202, -201, -204/197, -203, and -196) and nona- PBDEs (BDE-208, -207, and -206). Uptake of -209 from food was estimated at only 3.2% (*22*). Although rainbow trout are from the Family *Salmonidae* and sunfish are *Centrachidae*, the same PBDEs (hepta- through deca-PBDEs) were detected (Figure 3b). Similar nona- through hepta-PBDEs were also detected in the 2005 sunfish sample, but -209 was not observed. Although it is likely exposure conditions varied between the in-lab -209-exposure study and at the Roxboro outfall, congener profiles for the trout and sunfish (carp and chub) were similar, suggesting analogous metabolic pathways. Stapleton et al. (*22*) confirmed that -209 debromination was performed by the fish itself by using a preparation of both trout and carp liver microsomes (*22*). These findings support the current study's conclusion that -209 is bioavailable and can undergo metabolic debromination in the field, resulting in the production of lower brominated PBDEs. Hence continued deca-BDE use may lead over time to increased PBDE burdens in organisms living in aquatic environments and terrestrial animals.

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Supporting Information Available

Additional details and Tables ST1, ST2 and ST3. This material is available free of charge via the Internet at http://pubs.acs.org.

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